

(19) World Intellectual Property  
Organization  
International Bureau



09 JUN 2005

(43) International Publication Date  
24 June 2004 (24.06.2004)

PCT

(10) International Publication Number  
WO 2004/052925 A3

(51) International Patent Classification<sup>7</sup>: C12N 15/31,  
C07K 14/285, 16/12, C12N 5/10, G01N 33/50, A61K  
39/102

Imperial College London, Exhibition Road, London SW7  
2AZ (GB).

(21) International Application Number:  
PCT/GB2003/005349

(22) International Filing Date: 8 December 2003 (08.12.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
0228691.2 9 December 2002 (09.12.2002) GB

(71) Applicant (for all designated States except US): IM-  
PERIAL COLLEGE INNOVATIONS LIMITED  
[GB/GB]; Electrical and Electronic Engineering Building,

(72) Inventors; and

(75) Inventors/Applicants (for US only): KROLL, John,  
Simon [GB/GB]; Imperial College Innovations Limited,  
Electrical and Electronic Engineering Building, Imperial  
College London, Exhibition Road, London SW7 2AZ  
(GB). LANGFORD, Paul, Richard [GB/GB]; Imperial  
College Innovations Limited, Electrical and Electronic En-  
gineering Building, Imperial College London, Exhibition  
Road, London SW7 2AZ (GB). BOSSE, Janine [CA/CA];  
Imperial College Innovations Limited, Electrical and  
Electronic Engineering Building, Imperial College London,  
Exhibition Road, London SW7 2AZ (GB). BEDDEK,  
Amanda [GB/GB]; Imperial College Innovations Limited,  
Electrical and Electronic Engineering Building, Imperial  
College London, Exhibition Road, London SW7 2AZ  
(GB). RYCROFT, Andrew [GB/GB]; Imperial College

[Continued on next page]

(54) Title: ACTINOBACILLUS PLEUROPNEUMONIAE VIRULENCE GENES

Class	Strain	Gene	Biochemistry Data		Accession or Patent Number	Name of Putative Product	In vitro Cl.	In vivo Cl.	
			%	Spms					
Cell Surface	8C3	gblC			AAC00191	cellular polysaccharide repeat	1.839	3.88E-03	
	28A9		10B (p4)	27D3	62	AAP2181	LPS or capsule biogenesis		1.869
	9B7	gblJ				AAC28128	LPS core biogenesis		1.739
	22B7	gblJ				AAC04894	LPS O-acetyl biogenesis		0.2044
	10B11	hucC	hucC (p4)	10B	15	AAC04849	LPS O-acetyl biogenesis		0.233
	132B	hucC				AAC04842	LPS O-acetyl biogenesis		0.234
	21B8	hucP				AAC04843	LPS O-acetyl biogenesis		0.233
	15A8	hucP	hucP (p4)	27D3	27B	g11897178	LPS O-acetyl biogenesis		0.739
	4D4	gblB (p4)	08D3	85	AAC00089	Exopolysaccharide	1.879		
	17A4	hucP2 (p4)	08D3	257	04821		Outer membrane protein P2 precursor		1.149
Metabolism	10B12	hucP (p4)	01D7	83	P44584	ADP-kinase synthetase	1.629	3.43E-04	
	10A11	hucP (p4)	05D8	34	AAC23273	phosphotransferase	0.802		
	13C7	hucA (p4)	08D3	124	P43714	ATP synthase	0.214		
	17B8	hucH (p4)	03D7	188	P46108	cytochrome C biogenesis	1.160		
	20D11	hucA (p4)	03D7	183	P31316	transmembrane biogenesis	1.849		
	20C3	hucA (p4)	01D4	394	CAAT1482	outer membrane	0.849		
	13B	hucA (p4)	07D4	114	P45211	outer membrane	1.160		
	13C6	hucA				AAC00089	ATPase		0.239
	23B11	hucB (p4)	08D3	77	AAC00081	polysaccharide repeat	1.289		1.8E-04
	10B	hucP (p4)	78D7	123	AAP00421	phosphotransferase	1.139		
0A7	hucB (p4)	08D7	429	P43819	transmembrane	0.739			
23C9	hucA (p4)				AAC00081	transmembrane	0.739		
28A10	hucC (p4)	73D1	343	P43828	proteinase	0.479			
28B12	hucB (p4)	01D7	71	P44712	transmembrane	0.839			
28A10	hucC (p4)	08D3	188	P44823	transmembrane	0.867			
0P6	hucB (p4)	04D4	258	CA1016	transmembrane	0.489			
27A12	hucB				P47818	transmembrane	0.502		
28C6	hucC (p4)	08D3	230	AAC00081	transmembrane	0.839	1.8E-04		
15A11	hucC (p4)	08D3	43	AAC00081	transmembrane	0.839			
28A6	hucA (p4)	78D4	127	P44828	transmembrane	0.839			
23B7	hucA (p4)	77D3	157	P44828	transmembrane	0.744			
28B12	hucA (p4)	03D3	43	P44828	transmembrane	0.839			
0C12	hucA (p4)	78D3	139	P47123	transmembrane	0.837			
28C3	hucA (p4)	08D3	119	P44828	transmembrane	0.839			
18B10	hucA (p4)	03D3	81	AAC00081	transmembrane	0.839			
21D3	hucA (p4)	01D3	189	P44720	transmembrane	0.749			
Signal									

(57) Abstract: An attenuated *Actinobacillus pleuropneumoniae* bacterium has a mutation in a gene required for bacterial virulence. Vaccines based upon the bacterium are provided, as are isolated virulence genes and polypeptides and uses thereof.



Innovations Limited, Electrical and Electronic Engineering Building, Imperial College London, Exhibition Road, London SW7 2AZ (GB). SHEEHAN, Brian [IE/IE]; Imperial College Innovations Limited, Electrical and Electronic Engineering Building, Imperial College London, Exhibition Road, London SW7 2AZ (GB).

(74) Agents: MACLEAN, Martin, Robert et al.; Mathys & Squire, 100 Gray's Inn Road, London WC1X 8AL (GB).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

(88) Date of publication of the international search report:

4 November 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/GB 03/05349

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/31 C07K14/285 C07K16/12 C12N5/10 G01N33/50  
A61K39/102

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, Sequence Search

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WO 00/61724 A (PHARMACIA &amp; UPJOHN, INC.) 19 October 2000 (2000-10-19) cited in the application the whole document see especially: SEQ ID NOS: 140, 141 ORF ID: exbB mutant ID: AP11E7 page 42 - page 53; examples 7-11; tables 2-4 and page 233 - page 234</p> <p>----- -/--</p>	<p>1-13, 16-35</p>

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

21 June 2004

Date of mailing of the international search report

14 09. 2004

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Fuchs, U

## INTERNATIONAL SEARCH REPORT

Internal Application No  
PL 1/GB 03/05349

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02/075507 A (PHARMACIA & UPJOHN COMPANY) 26 September 2002 (2002-09-26) the whole document see especially: SEQ ID NO: 140, 141 ORF ID: exbB mutant ID: AP11E7 page 45 - page 57; examples 7-11; tables 2-4 and page 234 - page 236	1-13, 16-35
X	----- ELKINS, C. ET AL.: "Role of the Haemophilus ducreyi Ton System in Internalization of Heme from Hemoglobin" INFECTION AND IMMUNITY, vol. 66, no. 1, January 1998 (1998-01), pages 151-160, XP002285338	16-27, 30-33
A	the whole document  see especially: page 154 - page 155; figure 2 ExbB, ExbD, TonB proteins and page 158, column 2, line 26 - line 37	1-13,28, 29,34,35
A	----- FULLER, T.E. ET AL.: "A genetically-defined riboflavin auxotroph of Actinobacillus pleuropneumoniae as a live attenuated vaccine" VACCINE, vol. 18, no. 25, 15 June 2000 (2000-06-15), pages 2867-2877, XP004203577 the whole document	1-13, 16-35
T	----- BEDDEK, A.J. ET AL.: "Two TonB Systems in Actinobacillus pleuropneumoniae: Their Roles in Iron Acquisition and Virulence" INFECTION AND IMMUNITY, vol. 72, no. 2, February 2004 (2004-02), pages 701-708, XP008031967 the whole document -----	1-13, 16-35

# INTERNATIONAL SEARCH REPORT

tional application No.  
PCT/GB 03/05349

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☒ Claims Nos.: 14, 15, 36-40 (completely) and 17, 18, 29-33 (partially)  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:  
see FURTHER INFORMATION sheet PCT/ISA/210
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
  
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
  
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
1-13, 16-35 (partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 14, 15, 36-40 (completely) and 17, 18, 29-33 (partially)

Present claims 14 and 15 and dependent claims 17, 18, 29-33 relate to a polynucleotide defined by reference to a desirable characteristic or property, namely, for claim 14: "encoding a gene product which is not naturally found in *A. pleuropneumoniae*, but whose expression therein is capable of modulating ... the virulence of that bacterium", and for claim 15: "which is not naturally found in *A. pleuropneumoniae* but which is capable of modulating the virulence of that bacterium by its direct interaction with *A. pleuropneumoniae* virulence genes or gene products".

The claims cover all polynucleotides having this characteristic or property, whereas the application provides no support within the meaning of Article 6 PCT and no disclosure within the meaning of Article 5 PCT for such polynucleotides. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the polynucleotide by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search impossible. Consequently, no search has been carried out for claims 14 and 15 and for dependent claims 17, 18, 29-33.

Furthermore, present claims 36, 38-40 relate to an anti-bacterial agent defined by reference to a desirable characteristic or property, namely "identified by the method of claims 34 or 35".

The claims cover all compounds having this characteristic or property, whereas the application provides no support within the meaning of Article 6 PCT and no disclosure within the meaning of Article 5 PCT for such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search impossible. Consequently, no search has been carried out for claims 36, 38-40.

The same applies to claim 37 relating to a "method of modulating the transcription of such virulence genes through the use of oligonucleotide-directed triplet helix formation". However, the application provides no support within the meaning of Article 6 PCT and no disclosure within the meaning of Article 5 PCT for such oligonucleotides. Accordingly, no search has been carried out for claim 37.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

search report has been established need not to be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-13, 16-35 (partially)

An attenuated *Actinobacillus pleuropneumoniae* bacterium having a mutation in a gene required for bacterial virulence which comprises the nucleotide sequence of SEQ ID NO: 1, a composition containing said attenuated *A. pleuropneumoniae* bacterium, use of said attenuated *A. pleuropneumoniae* bacterium in the manufacture of a medicament for preventing or alleviating an infection of an animal with *A. pleuropneumoniae*, an isolated polynucleotide comprising a) a nucleotide sequence of SEQ ID NO: 1, b) a nucleotide sequence encoding the polypeptide which is encoded by the nucleotide sequence recited in a), c) a nucleotide sequence which hybridizes to the nucleotide sequence of a) and/or b) or to its complement under conditions of moderate to high stringency, d) a fragment of any one of the nucleotide sequences of a)-c) which fragment retains an immunological properties and/or biological activity of the recited nucleotide sequence, a vector comprising said polynucleotide, a host cell comprising said vector, an isolated *A. pleuropneumoniae* polypeptide encoded by said polynucleotide, a method of producing said polypeptide, a composition containing said polypeptide, an antibody which specifically recognizes said polynucleotide or said polypeptide, a method for identifying an anti-bacterial agent which is capable of modulating the function of the said *A. pleuropneumoniae* virulence gene, an attenuated bacterium containing a mutation in a gene comprising a nucleotide sequence which is capable of hybridising to the nucleotide sequence defined by SEQ ID NO: 1 under consitions of moderate to high stringency, a composition containing said attenuated bacterium, use of said attenuated bacterium in the manufacture of a medicament for the therapeutic treatment or prophylactic protection of an animal against infection by the corresponding wild-type bacterium, an isolated virulence polypeptide encoded by said gene, a composition containing said polypeptide, an antibody which specifically recognizes said polynucleotide or said poypeptide;

---

2. claims: 1-13,16-35 (partially)

idem as subject 1, but limited to SEQ ID NO: 2;

---



# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/GB 03/05349

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 0061724	A	19-10-2000	AU 4077600 A	14-11-2000
			BR 0009663 A	09-04-2002
			CA 2366520 A1	19-10-2000
			CN 1351653 T	29-05-2002
			EP 1171577 A2	16-01-2002
			JP 2002541790 T	10-12-2002
			NZ 514883 A	26-03-2004
			WO 0061724 A2	19-10-2000
			US 2004110268 A1	10-06-2004
			ZA 200108262 A	08-01-2003
-----				
WO 02075507	A	26-09-2002	US 2004110268 A1	10-06-2004
			CA 2438315 A1	26-09-2002
			EP 1368456 A2	10-12-2003
			WO 02075507 A2	26-09-2002
-----				